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HISTOLOGICAL RESPONSES OF *VICIA FABA* TO INDOLEACETIC ACID

A DISSERTATION SUBMITTED TO THE FACULTY OF THE DIVISION OF THE BIOLOGICAL SCIENCES IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY
AUGUST, 1942

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HISTOLOGICAL RESPONSES OF VICIA FABA TO INDOLEACETIC ACID

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 548

BARBARA F. PALSER

(WITH TWENTY-THREE FIGURES)

Introduction

The gross and histological responses of several varieties of plants to various growth-regulating substances, applied laterally to intact and apically to decapitated stems, have been reported. A few recent papers (8, 10, 19) suggest that certain growth substances may induce polyploidy in nuclei of the treated organs. There is no evidence recorded for bean (17), *Iresine* (14), tomato (6), or *Lilium* (1) that indoleacetic acid does induce polyploidy. On the contrary, BEAL found evidence in *Lilium* indicating that all stages of mitosis were regular and chromosome structure normal throughout division.

For the present investigation, plants of *Vicia faba* were grown in pots containing garden soil under ordinary greenhouse conditions during the autumn, winter, and spring of 1941–1942. Following germination, the cotyledons and the first or cotyledonary node of *Vicia* remain underground; small bracts are present at the second and third nodes, and the first true leaf with its large stipules is at the fourth node. Stems were decapitated at the upper end of the fourth internode, just below the fifth node, at the time the second true leaf was beginning to expand and the fifth internode beginning to elongate slightly (fig. 1*A*). The cut surface was covered with a layer of a 1.5 per cent mixture of indoleacetic acid in lanolin or with lanolin alone.

The tips of the treated plants were collected at hourly intervals from 15 to 48 hours following treatment, and after that at 4-hour intervals up to 104 hours. Others were allowed to grow for a month or more before collection. The tips were killed and fixed in Navashin's solution, and imbedded according to the butyl alcohol-paraffin method. Sections were cut at 10 μ and stained in a modified triple stain.

Plants of a series grown in late spring and early summer, 1942, and treated as before, were collected at intervals, sectioned freehand, and stained with iodine-potassium iodide solution. These sections showed the effects of treatment on starch stored in the endodermis and on the chloroplasts present in the outer cortex.

Observations

GROSS RESPONSES

During the first 24 hours after treatment little or no change could be observed. Occasionally, if the tissue when treated was in a younger, more immature stage than usual, a very slight swelling could be seen. During the next 24 hours the swelling of the tip, although never very great, became distinct. The bright green of the stem became paler in the swollen region. The amount of swelling increases with length of time after treatment (fig. 1*C–F*). The plants treated with lanolin alone showed no similar

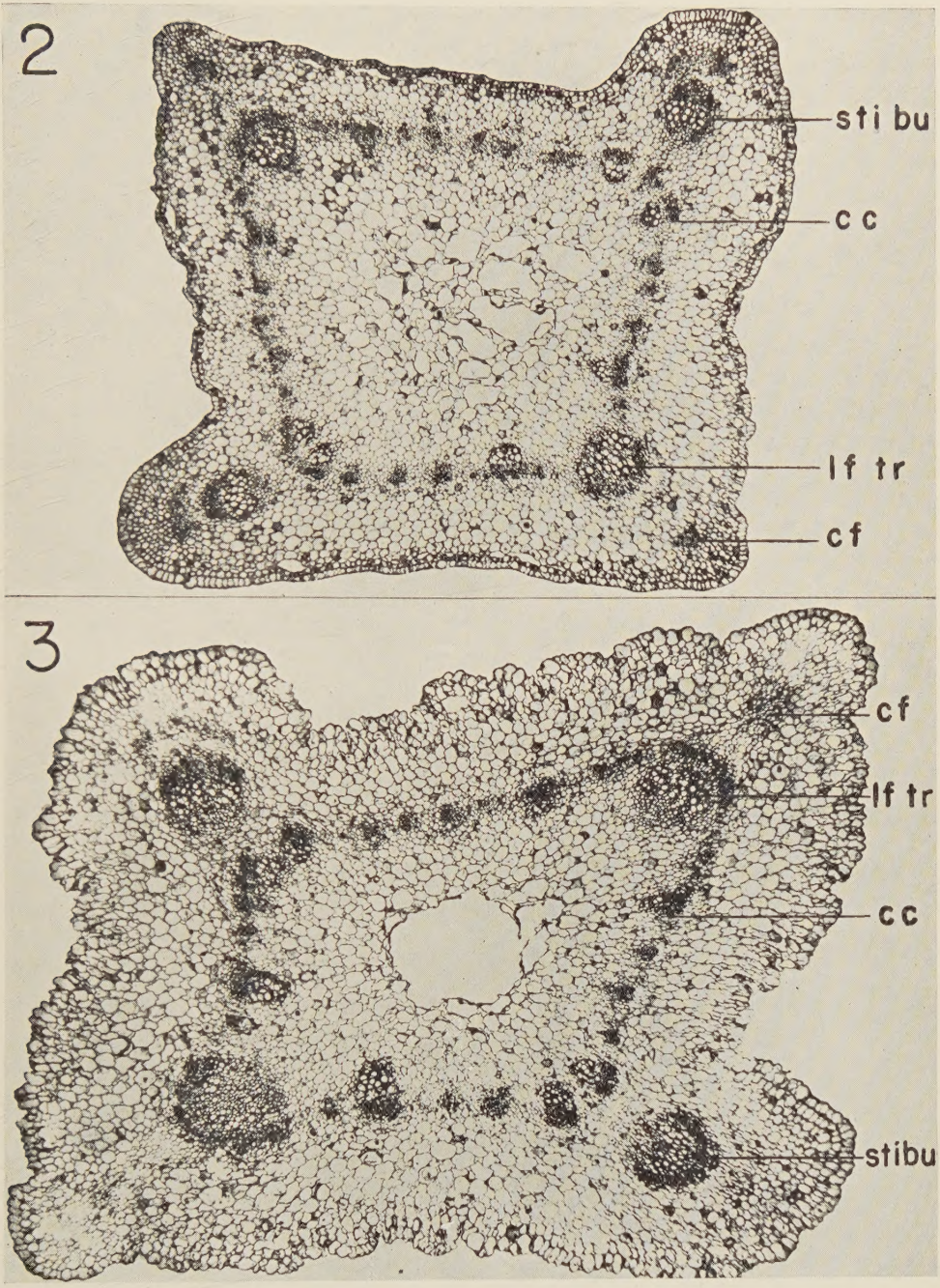
swelling or decrease in color (fig. 1*B*), and on them lateral shoots developed rapidly at the second and third nodes. Shoots also developed on plants treated with indoleacetic acid but were slower in starting and somewhat slower in later development. The tumors that had developed for a month or more showed a slightly darkened, roughened surface, and some showed a small amount of callus proliferation. Plants decapitated and left untreated showed a dry exposed surface but otherwise little change over a considerable period of time. The relative size of tumors formed on stems of *V. faba* following treatment was much smaller than those formed on bean in a corresponding length of time. A young tumor may extend 2–3 mm. back from the cut surface and the fading of color slightly farther. Old tumors do not extend much farther back in the stems than young ones, the longest extending 3–5 mm. The increase in diameter varies from an amount barely visible to once and a half or twice the original diameter.

FIG. 1.—*A*, *V. faba* at time of treatment, showing region of decapitation. *B*, decapitated plant treated with lanolin alone, 94 hours after treatment. *C–F*, decapitated plants treated with 1.5 per cent indoleacetic acid in lanolin (*C*, 30 hours after treatment; *D*, 71 hours; *E*, 94 hours; *F*, 30 days after treatment).

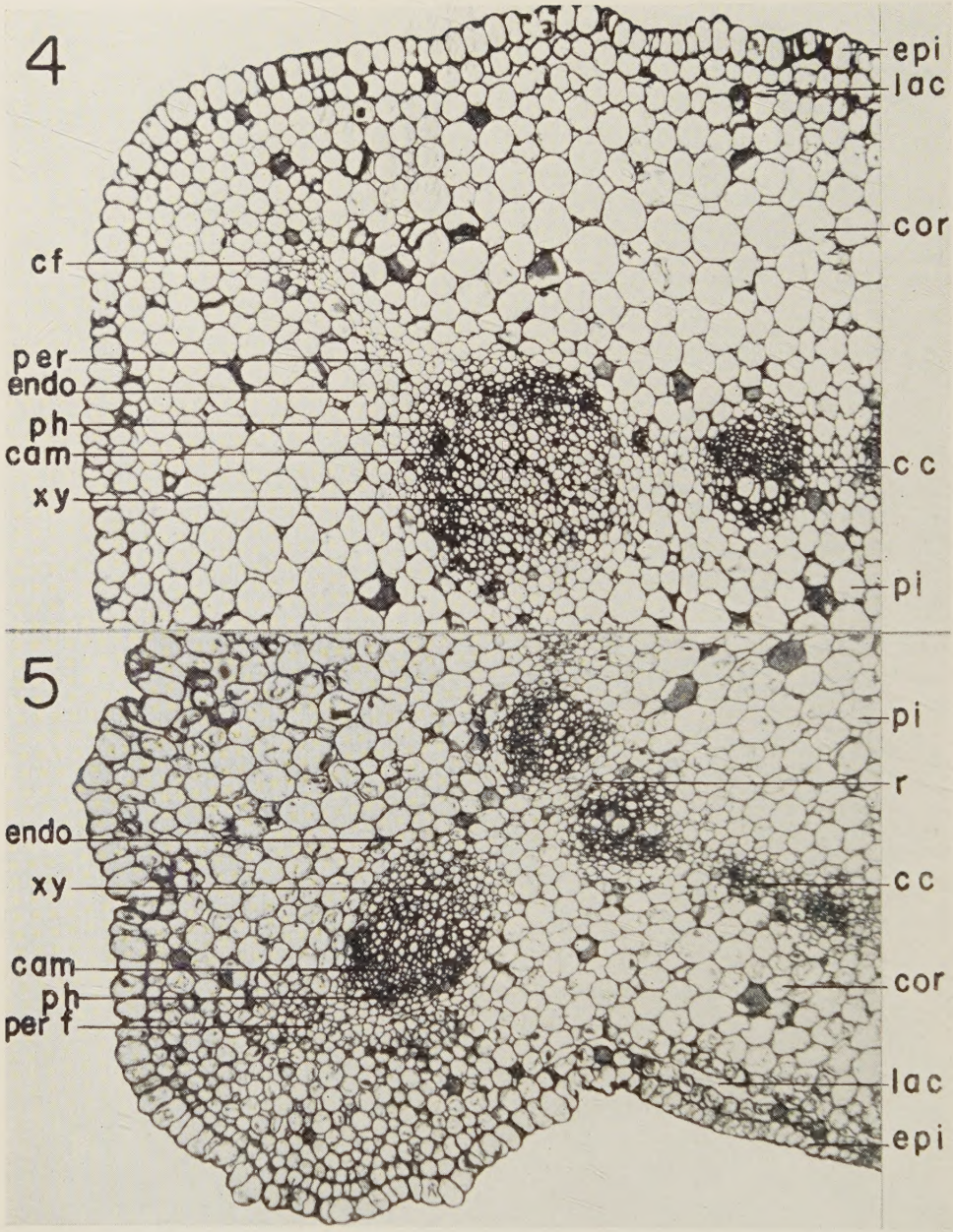
HISTOLOGICAL DETAILS

The transition region in *V. faba* extends through the first two internodes and is in many respects similar to that in pea (**7, 24, 15**). Typical stem anatomy is present in the treated fourth internode. At the time of treatment many of the cells are still immature, but the regions are differentiated and can be easily distinguished. Figure 2 shows a cross-section of the fourth internode 32 hours after treatment with lanolin alone. A central cylinder of vascular bundles is evident, with two polar bundles much more prominent than the others, the leaf traces for the leaves at the fifth and sixth nodes. In the cortex outside each trace is a small bundle of fibers which departs into the leaf with the leaf trace. Such a leaf trace and cortical bundle are shown in figure 4. The leaf gap is closed by the adjacent bundles of the central cylinder. Large fibrovascular bundles are also present in the cortex in the corners alternate with those in which the leaf traces are situated. These are the stipular bundles (fig. 5). At the fifth node each stipular bundle divides into two, one-half supplying the stipule and the other half remaining in the cortex through the fifth internode. The bundles of the central cylinder vary in size and are separated by parenchymatous rays of differing widths. Rays one cell in width are formed in the secondary xylem.

Surrounding the vascular cylinder and the stipular bundles is an endodermis,



FIGS. 2, 3.—Cross-sections of fourth internode of *V. faba* just below fifth node. Fig. 2, 0.85 mm. from cut surface; 32 hours after treatment with lanolin alone. Fig. 3, 0.85 mm. from surface; 31 hours after treatment with 1.5 per cent indoleacetic acid in lanolin. *sti bu*, stipular bundle; *lf tr*, leaf trace; *cf*, cortical strand of fibers; *cc*, central cylinder.



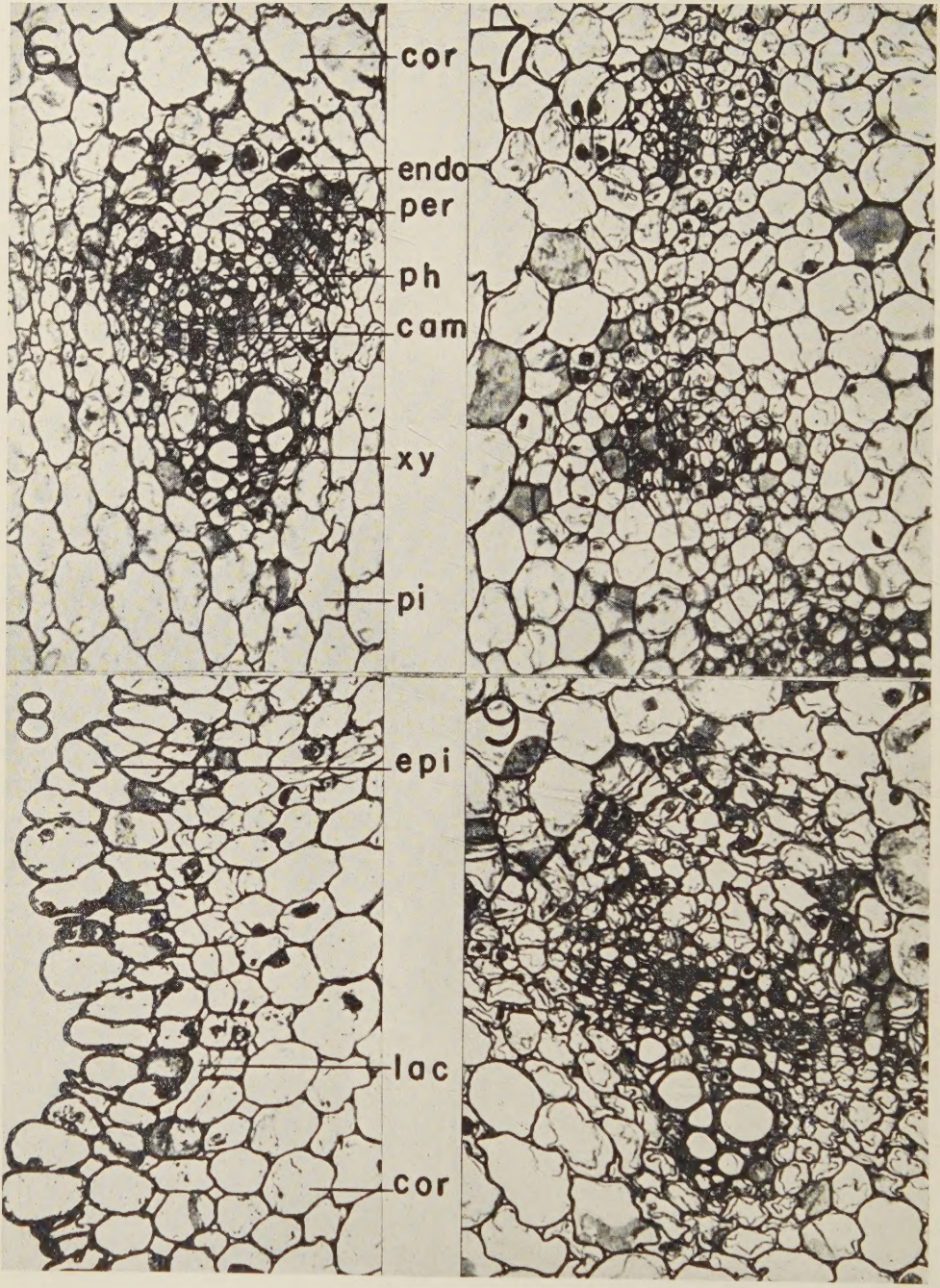
FIGS. 4, 5.—Fig. 4, 0.6 mm. below surface; portion showing leaf trace and cortical strand of fibers 96 hours after treatment with lanolin alone. Fig. 5, 0.9 mm. from surface; portion showing cortical stipular bundle 56 hours after treatment with lanolin alone. *epi*, epidermis; *lac*, lacuna; *cor*, cortex; *pi*, pith; *per*, pericycle; *per f*, pericyclic fibers; *endo*, endodermis; *ph*, phloem; *cam*, cambium; *xy*, xylem; *r*, ray.

the cells of which are not clearly differentiated but which is easily recognizable in fresh sections stained with iodine because of the presence of stored starch. Associated with each larger bundle is a small group of pericyclic fibers, largely immature, often separated from the phloem by a few parenchymatous cells. A fascicular cambium is usually present in the larger bundles but not always in the smaller ones. With increased age a fascicular cambium develops in the smaller bundles, and an interfascicular cambium, which may give rise to a few derivatives, forms between the bundles. The cortex consists of parenchymatous cells, rounded in cross-section, with intercellular spaces. The outer four or five layers of cells contain chloroplasts. In the outer part of the cortex between the first and second layers under the epidermis small lacunae are present. The epidermis is made up of rather small cells, rounded or radially rectangular in cross-section, and is covered with a thin layer of cutin. It contains numerous stomata, and there are a few glandular hairs. The cells of the pith are parenchymatous and rather large. Usually there is complete disintegration of the innermost ones, leaving the stem hollow.

The initial changes following treatment with indoleacetic acid are so gradual that it is difficult to determine when they first begin. By 30 hours after treatment, however, certain characteristic differences can be seen (fig. 3). The epidermal cells and outer layers of the cortex have enlarged in radial dimension. Chloroplasts have almost entirely disappeared from the enlarged cells. The enlargement causes the margin of the stem, which in an untreated plant or in one treated with lanolin alone is regular in outline, to be thrown into convolutions. At the same time the cells of the cambium have become somewhat more active. The region of undifferentiated derivatives is slightly wider, and the nuclei appear larger. In the parenchymatous cells of the cortex, rays, and pith, activity appears to be somewhat increased, as some of the nuclei are larger and there are more division figures observable than in a stem treated with lanolin alone for the same number of hours.

Development subsequent to this beginning is shown in figures 6–23. Although plants were collected at frequent intervals following the appearance of early differences, only those were photographed which showed a definite increase in response. All plants did not respond at exactly the same rate. Differences can be associated with the amount of lanolin mixture spread on the surface, with the maturity of the tissues at the time of treatment, and probably also with growing conditions at that time. Changes were initiated sooner and development proceeded more rapidly in those plants that were highly vegetative at the time of decapitation.

The changes observable at 30 hours increase with length of time after treatment, both in amount of response in regions already referred to and in number of regions responding. At 36 hours some of the endodermal cells have increased slightly in size, but more striking than this is the increase in size of the nuclei and the initiation of their division (fig. 6). An occasional endodermal cell divides in a tangential plane. In the region where this activity begins, just below the cut surface, starch has disappeared but is still present farther away from the surface, where activity in the endodermis has not yet begun. Early divisions of the endodermis always appear in connection with a vascular bundle, either large or



FIGS. 6–9.—Fig. 6, 0.95 mm. from surface; 36 hours after treatment, showing enlarged nuclei of first division of endodermis outside a bundle. Fig. 7, 1.05 mm. below surface; 52 hours after treatment, showing divisions in endodermis, formation of interfascicular cambium, and increase of cytoplasm. Fig. 8, 0.4 mm. from surface; 38 hours after treatment, showing tangential divisions in outer cortex. Fig. 9, 0.8 mm. below surface; 92 hours after treatment, showing repeated divisions in two planes within one endodermal cell forming provascular strand.

small. After 40 hours one division and occasionally a second have occurred in cells around the bundles; after 48 hours almost all the endodermal cells over bundles have begun to divide (fig. 7); after 72 hours the greater part of the derived endodermal layer is three or four cells thick; while at 80 hours divisions are beginning in the endodermal cells between the bundles, forming an almost continuous region of proliferated tissue around the stem. The extensions of the endodermis around the leaf traces, whether including the cortical fiber bundle or not, are evident (fig. 10). Between 84 and 92 hours the derivatives of one, sometimes two, endodermal cells undergo a series of divisions in various planes without subsequent enlargement, forming a compact group of small, very active cells (fig. 9). They appear to be provascular strands. The endodermis and cortex around the cortical fiber bundle divide, at first tangentially and later in several planes, forming provascular strands.

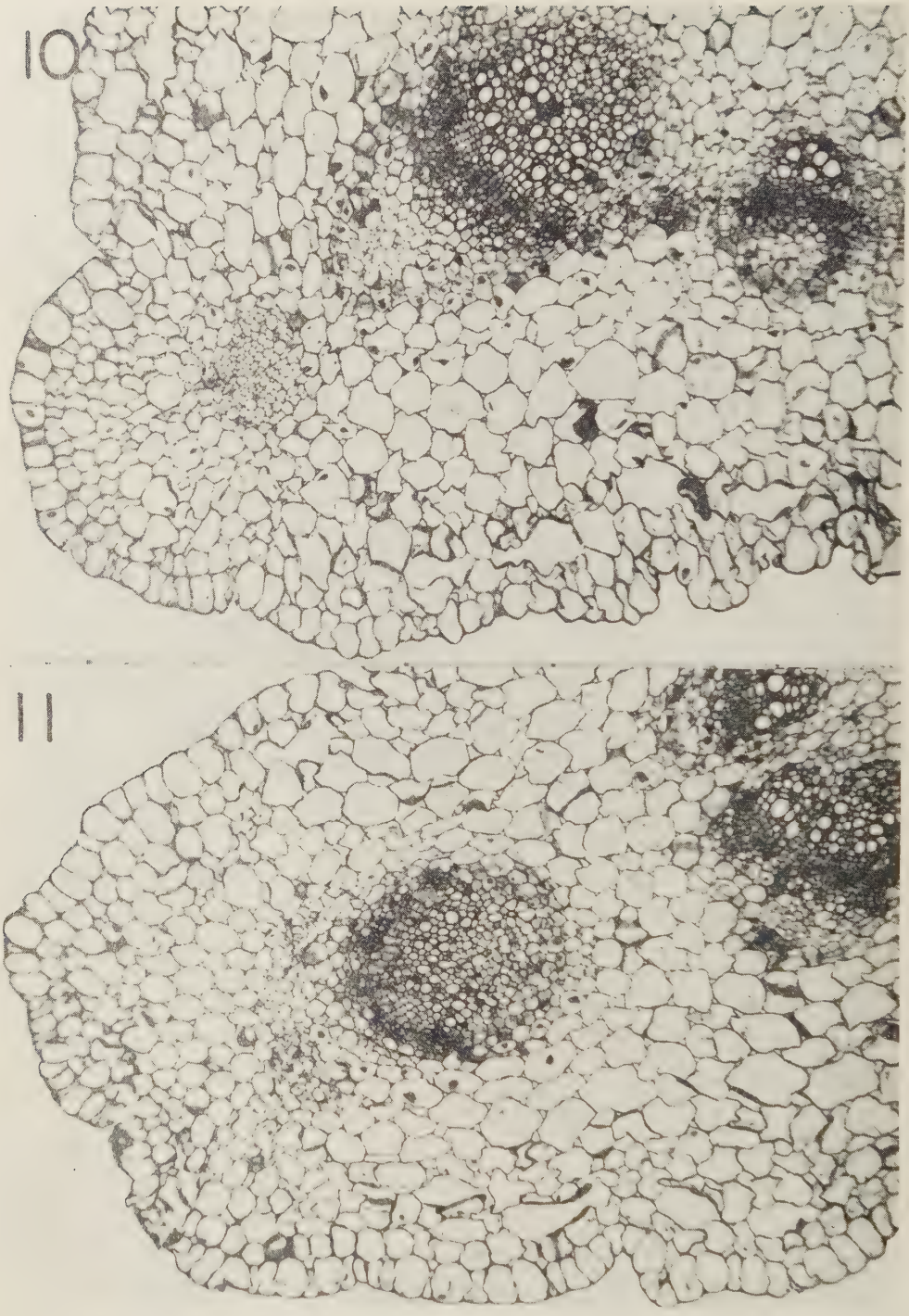
In a longitudinal section of a tumor 72 hours old the endodermis can be followed from a layer one cell wide at the surface to a layer three, four, or five cells wide immediately below the surface, and then narrowing again below, but more gradually, to a single layer. Portions of the endodermal cells sometimes appear to project into the pericyclic fibers next to them, similar to the projections of parenchymatous cells into vessels forming tyloses. Nuclei migrate into the projections and there undergo division.

After 40 hours, in some places in a few stems tangential divisions can be seen in the second cell layer of cortical parenchyma under the epidermis (fig. 8). This type of division does not appear at all consistently but is found occasionally in tumors of varying ages. It may perhaps be associated with the presence of a little lanolin mixture on the side of the stem as well as on the surface. In spite of care to put the mixture only on the decapitated surface, occasionally a little may get on to the side.

The amount of cytoplasm in the active cells tends to increase gradually after 36 hours but never becomes as dense as it does in bean. Activity in the cambium increases with length of time after treatment, older stems showing a noticeable development of secondary xylem. At 40 hours there are indications of the beginning of an interfascicular cambium, which become more evident after 48 hours (fig. 7). At 72 hours it is rather well developed, and at 80 hours its activity has almost completed the vascular cylinder (fig. 10).

By 48 hours after treatment cells scattered through cortex and pith show nuclei in various stages of division, together with cells which appear to have recently divided. These scattered divisions are chiefly transverse as seen in longitudinal section. Transverse divisions would account for the rather large number of mitotic nuclei observed in cross-sections, in contrast to the relatively small number of recently divided cells observed in cross-sections of even older tumors (up to 100 hours). They would also account for the increase in number of nuclei visible in cross-section of a treated stem, as the resulting cells are shorter in longitudinal dimension.

Parenchymatous cells of the phloem may show slight activity after 48 hours. Ray cells sometimes divide, particularly transversely, in the early stages. A longitudinal section may show some cells with two or three nuclei and no cross walls, but this is rather infrequent. Cambium, xylem, phloem, and ray parenchyma are active in early callus proliferation when callus is formed. By 72 hours in those tu-



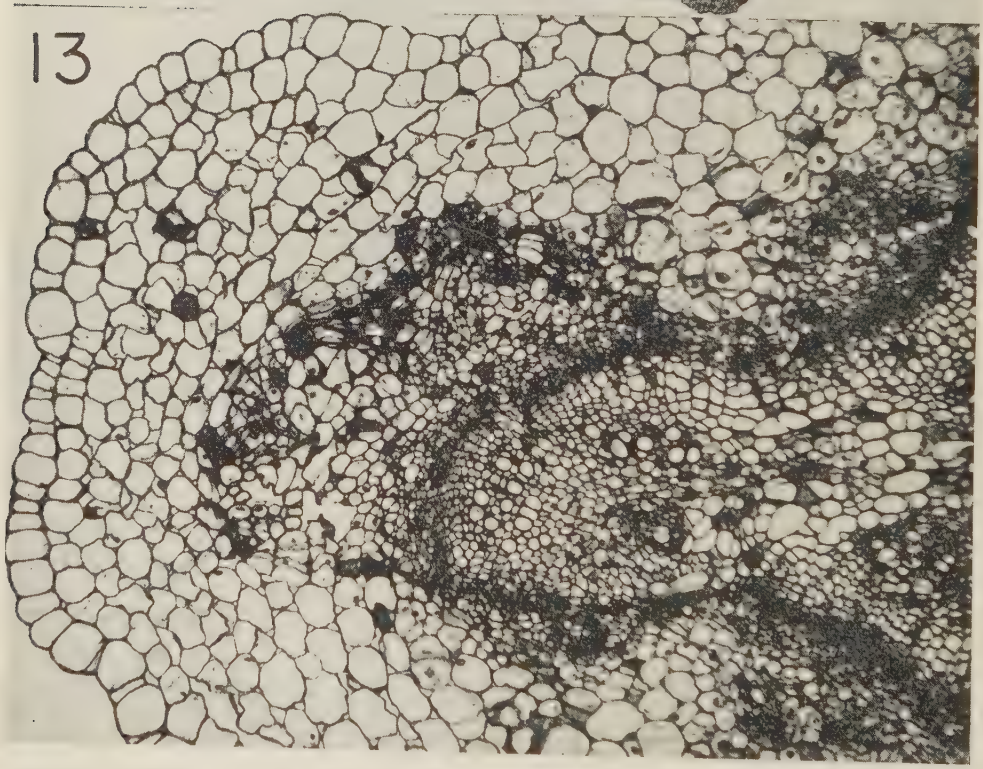
FIGS. 10, 11.—Seventy-two hours after treatment. Fig. 10, 1.1 mm. below surface; activity in endodermis around leaf trace; cortical strand of fibers outside central cylinder. Fig. 11, 1.6 mm. below surface; stipular bundle becoming active at inner edge.

mors which do form callus the living cells just below the cut surface have begun to elongate upward and to divide, beginning the formation of a callus over the surface. In much older tumors other parenchymatous cells also become active and add to the proliferation.

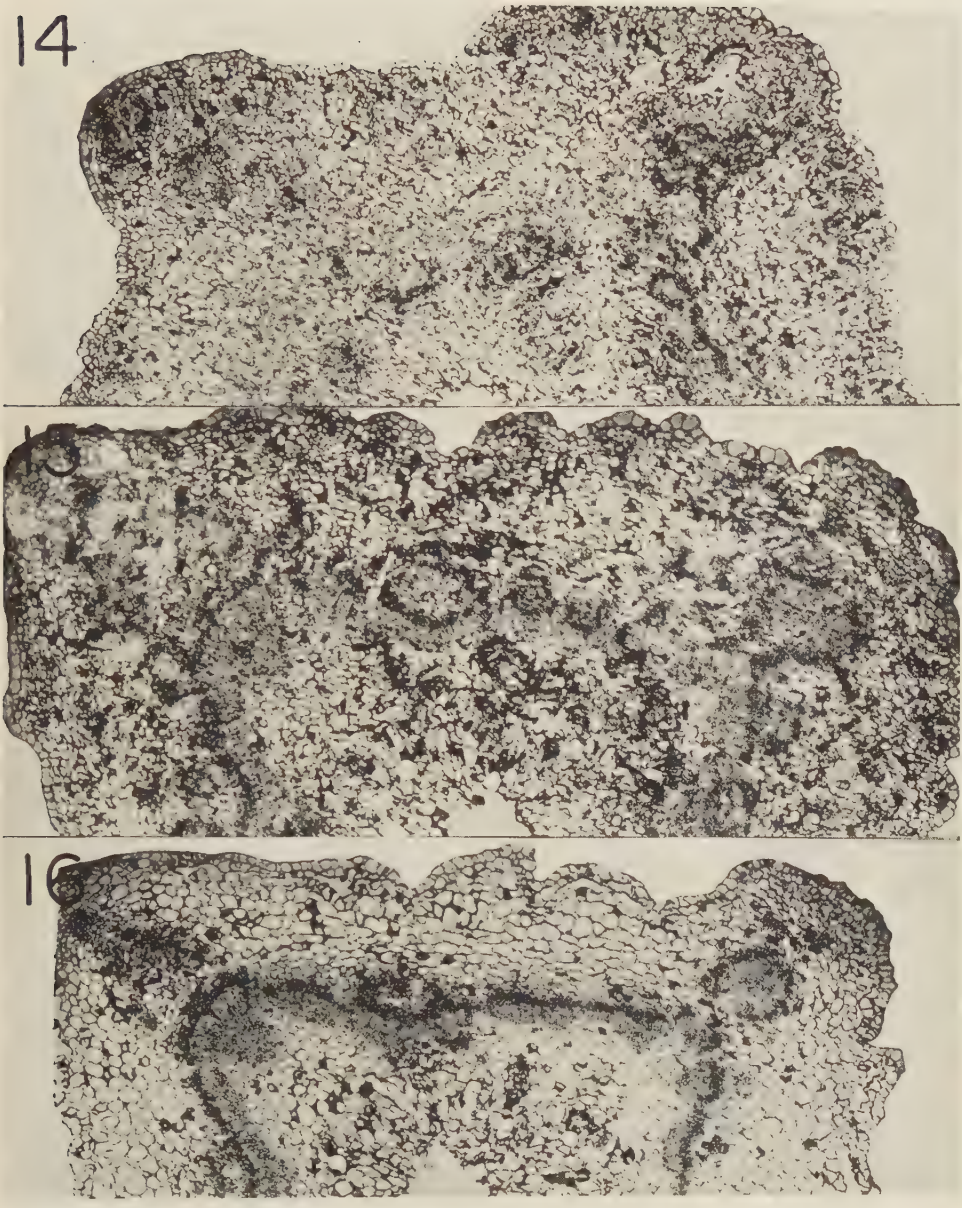
By 72 hours parenchymatous cells at the lateral and inner edges of the stipular bundles have become active and show several derivatives (fig. 11). Later such cells between the stipular bundle and the central cylinder may divide. None of the active regions extends more than 2–3 mm. back from the cut surface. The activity in the fascicular and interfascicular cambium, the enlargement of the cortical and epidermal cells, and occasionally the divisions of the endodermis appear farthest from the surface. The largest part of the tumor up to 100 hours after treatment is due to the radial enlargement of the cortex and epidermis.

Development of tumors subsequent to the first 100 hours has not yet been followed closely, but later responses will be described briefly from several older tumors of widely separated ages. After 7 days, responses already described have increased. Owing to the continued activity of the fascicular and interfascicular cambium, the vascular cylinder is almost complete around the stem, with a great deal of secondary xylem (fig. 12). Activity of the cells around the stipular bundles, especially laterally, has resulted in a continuation of the cambium, with xylem differentiating on the inner side and phloem on the outer. This development leads to a bundle, horseshoe-shaped in outline, with secondary xylem completely surrounding the primary strand except for a small area nearest the stele (figs. 12, 13). The endodermis has continued activity and in many places shows small vascular strands starting to differentiate. In some places the cortical cells next to the endodermis show an increase in both cytoplasm and divisions (fig. 13). Some of the pericyclic fibers, evidently immature at time of treatment, and some pericyclic parenchymatous cells, have become active and may show several derivatives. The walls of others of the fibers have thickened and remain single, or in small groups, scattered among the active cells. In one or two instances there are a few cells in the cortex, grouped together, which have divided repeatedly to form provascular masses. At this time, however, the largest part of the cortex is unresponsive to the treatment. The pith, which earlier showed little response, has become highly active. The parenchymatous cells bordering the hollow center have proliferated inward, forming a callus in the hollow portion. The cells of the callus and those next to it have divided repeatedly, in many places tangentially and in others in various directions, to form provascular masses.

Tumors a month or more old show a still greater response. A short distance below the cut surface is found the region of greatest activity (fig 15). The surface of application may be covered with a thin layer of callus, in which wound tracheids may have differentiated irregularly (fig. 14). In the active region the proliferated endodermis has given rise to vascular strands. Some cells of the pericycle are active in central cylinder bundles and in stipular bundles. In connection with the leaf traces, cells of the primary phloem have become active, forming vascular strands to increase the amount of extra-vascular tissue already differentiated in this region from pericycle and endodermis. The strands derived from endodermis, pericycle, and phloem are distinct from one another but



FIGS. 12, 13. Seven days after treatment. Fig. 12, 0.65 mm. below surface; cross-section showing continuous vascular cylinder, large amount of secondary xylem, callus proliferation inward from pith cells, activity in endodermis, beginning activity in cortex (lower left corner). Fig. 13, 0.5 mm. from surface; same stipular bundle as in upper right in fig. 12 enlarged. Bundle does not usually become continuous with central cylinder as shown here. Note vascularization in endodermis, activity in pericycle, activity of inner cortex.



FIGS. 14–16.—Cross-sections at different levels of 35-day-old tumor. Fig. 14, 0.25 mm. from surface; largely callus. Fig. 15, 0.85 mm. from surface; activity of cortex, pith, and vascularization of endodermis. Fig. 16, 2.5 mm. below surface; evidences of activity at this level in pith, cambium, endodermis, and around stipular bundle.

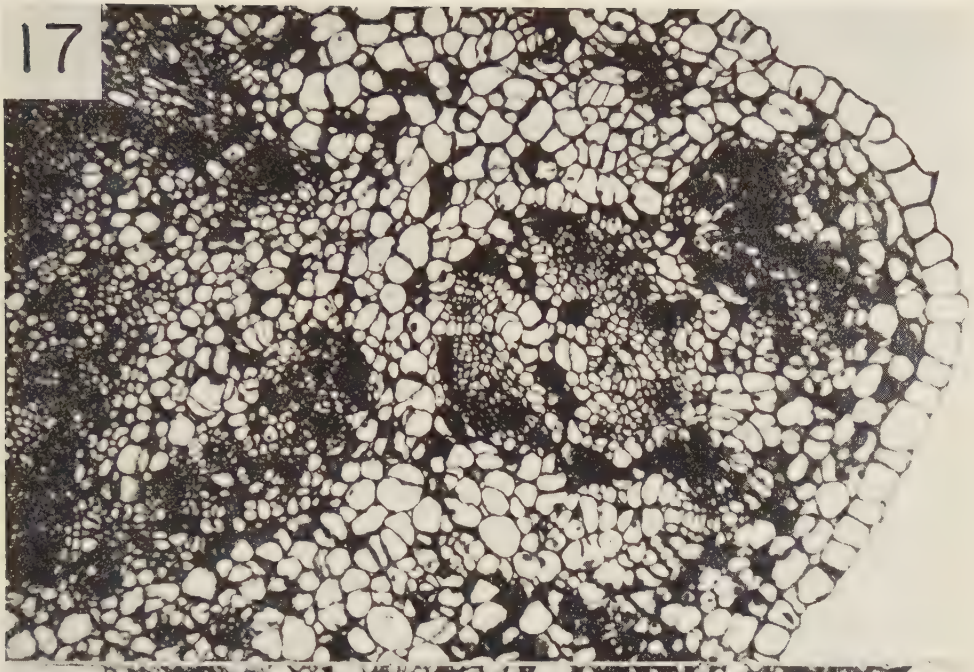
form a zone around the central vascular cylinder (figs. 15, 19, 20), around the stipular bundles joining them to the central cylinder (fig. 18), and around the large zone of pericycle outside the leaf traces (fig. 17). Many of the extra-vascular strands differentiate a cambium which is active in forming xylem toward the center of the stelar region and phloem to the outside. When the endodermis was continuous around the group of pericyclic fibers in the cortex, the extra-vascular strands derived from it are also continuous around the bundle. Strands found between the phloem and pericycle, evidently formed from the phloem, also develop a cambium, but differentiation of its derivatives is the reverse of that in the endodermal strands. Xylem is formed toward the pericyclic mass and phloem toward the phloem of the leaf trace. There is thus formed a complete new vascular ring, largely outside the main vascular cylinder (note leaf trace in the upper lefthand corner of fig. 15). Where the groups of fibers are separate from the leaf trace, the extra-vascular strands are between the cortical group and the leaf trace. The group of fibers, however, is surrounded by endodermis, which has formed—probably in conjunction with cortical parenchyma—vascular strands of the same type as around the leaf trace proper (fig. 17).

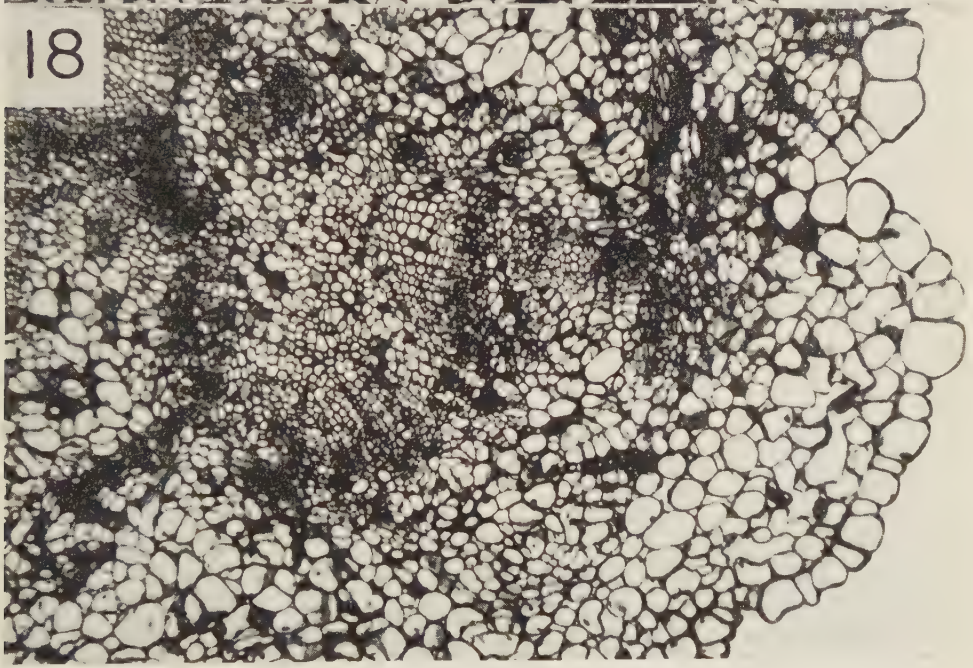
Cells of the cortex, both next to the endodermis and just under the epidermis, are active and may show as many as ten or more small cells within one mother cell wall. Vascular masses also differentiate in the cortex. Pith cells become active right up to the vascular bundles and may form vascular strands or masses of tracheids near the xylem of the main vascular cylinder (fig. 19). Callus in the hollow portion of the pith is extensive. Between 0.5 and 1 mm. from the treated surface hardly a single cortical or pith cell remains inactive, and small vascular and meristematic masses are numerous (fig. 15). Occasionally tangential divisions in the epidermis may be found.

Vascular strands in the endodermis (particularly in association with the leaf traces), the horseshoe development of the stipular bundles, the formation of callus from the pith cells, the activity of fascicular and interfascicular cambium with differentiation of secondary xylem, and the enlargement of cortical and epidermal cells—all extend farthest from the cut surface in the old tumors (figs. 16, 20). Division figures are scarce in old tumors. Apparently continued active proliferation has stopped.

In all the tumors cut, only three showed evidences of root formation. In only one were true root primordia located. This tumor had two primordia occurring at distances of 2 and 3 mm. below the cut surface. They show differentiation into epidermis, cortex, and stele and have a protective cap over the tip, but the histogenic region is not clear. They extend a short distance into the cortex from the vascular region (fig. 21). As these are the only organized roots found and no initial stages have been observed, it is extremely difficult to be sure from what tissue they are derived. As far as can be ascertained, they are formed in the region of a ray of the central cylinder. The endodermis may be involved in part in the formation, but probably the greater portion is derived from cells inward from it.

In addition to these organized root primordia, several peculiar structures were observed. In one tumor 7 days old one small and two large masses of proliferated tissue appear a short distance back from the treated surface. The masses consist of small cells with a great





FIGS. 17, 18.—Thirty-five days after treatment. Fig. 17, 0.85 mm. from surface; leaf trace showing separate vascularization of endodermis around cortical group of fibers. Note activity and groups of wound tracheids in cortex. From same section as fig. 15 from portion not shown in fig. 15. Fig. 18, 0.85 mm. from surface; enlargement of upper right corner of fig. 15; stipular bundle showing extra-vascular bundles joining bundle to central cylinder. Note activity of cortex.

FIG. 19.—Thirty-five days after treatment, 0.85 mm. below surface; enlargement of two small bundles shown in fig. 15. Small bundles of central cylinder showing extra-vascular strands of endodermis, activity of phloem, ray, and pith; thickenings on wound tracheids in pith.

FIG. 20.—Diagonal section of 35-day-old tumor through leaf trace, showing strand in endodermal region, proliferation in cortex, activity of phloem and pericycle, and both pith and surface callus.

deal of cytoplasm and large nuclei. The cells are in active process of division. The group of cells shows no organization of any kind. It has not been possible to determine their derivation accurately, but the endodermis appears to have been

FIG. 21.—Longitudinal section of root primordium 2 mm. from surface of 46-day-old tumor.

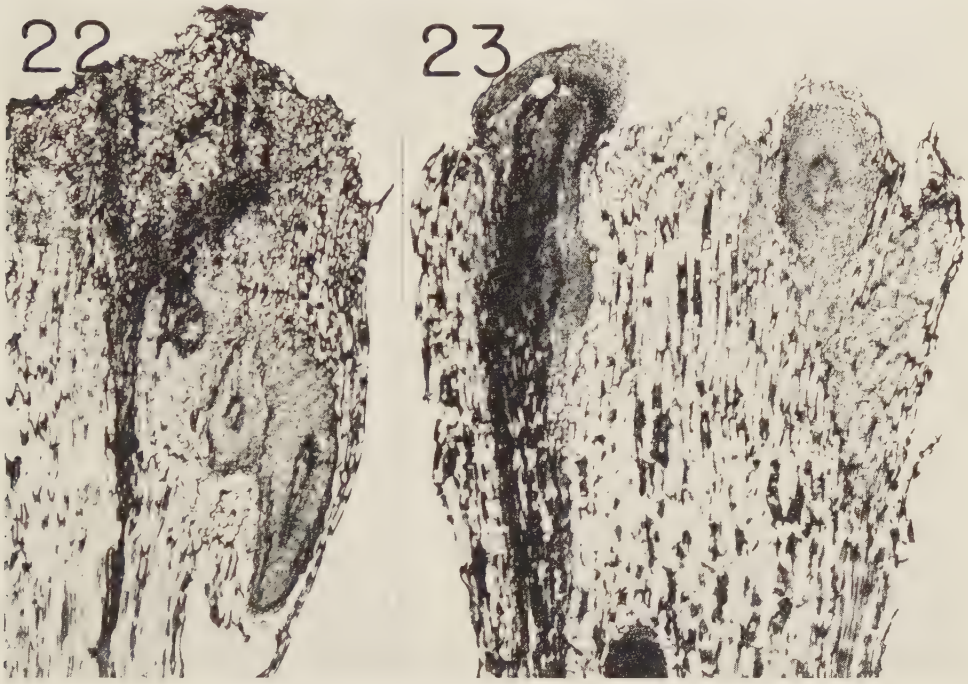
particularly active. In two older tumors proliferations having no particular organization were found. They show differentiated tracheids in the region adjacent to and in contact with the vascular strand of the stem; the differentiated tracheids are continued by regions of smaller cells almost to the edge of the proliferation. The cells are not meristematic; their cytoplasm is highly vacuolate. They may be derived from cells in close association with the leaf trace or stipular bundles, or from cells in the region of a ray of the central cylinder.

The shapes and sizes of the proliferations are various, but tend toward a rounded, somewhat elongated structure, with a suggestion of a ring of differentiated cells, which in many cases are vascular—at least near the bundle with which union is established. They extend upward above the callus surface (fig. 23), laterally through the cortex, or laterally and downward in the cortex (fig. 22). The interpretation suggested is that they may have originated as unorganized meristematic masses of tissue, as in the 7-day-old tumor. Differentiation took place in the direction of a root primordium but did not progress far. With later growth of the tumor the cells did not remain meristematic, but most of them enlarged, became vacuolate, and a few became lignified, finally resulting in the rather unorganized masses of tissue seen in the two old tumors.

CYTOLOGICAL DETAILS

Almost without exception, nuclei of the endodermis, pericycle, cambium, and parenchymatous cells of the vascular bundle and of the cortex and pith in close association with the vascular tissue, and, with occasional exceptions, other cells of the cortex and pith, are of normal size. If in division, they show the diploid number of chromosomes, 12. The normal resting nucleus has two medium-sized nucleoli which may fuse to form one large one. The stages of mitosis all proceed in a normal manner. In a few instances, as already stated, nuclear division apparently is not accompanied by cell plate formation, thus giving rise to binucleate and, in a very few cases, multinucleate cells. In some of the large par-

enchymatous cells of the pith and cortex of both treated and control stems (although somewhat more frequently in the treated) large nuclei are found which may have one very large nucleolus or two large or four medium-sized ones. These number of polyploid cells, the fact that they are found in controls as well as in treated stems, and the regions in which they occur, do not as yet indicate any particular significance to the polyploid condition observed in this experiment.



FIGS. 22, 23.—Proliferations in old tumors. Fig. 22, 35 days old, diagonal section; portions of three proliferations at different distances from stipular bundle. Fig. 23, 46 days old, tangential section; region of cortex, stipular bundle on left. Mass at right establishes union with leaf trace. Portion of cross-section of root primordium at base.

facts, according to BERGER (4), suggest a tetraploid condition. Division figures found in comparable cells show more than the diploid number of chromosomes. In a very few instances the degree of polyploidy may be greater, but this is highly infrequent. These cells with tetraploid nuclei are few, are found in regions not extremely active, and appear to occur normally in the stems of control plants to a certain extent. So few were found that it cannot be said by what method doubling occurred. The small

Discussion

The responses just discussed enlarge upon and corroborate to a certain extent the work of LAIBACH (19) and SOLACOLU *et al.* (27) on *Vicia faba*. LAIBACH reports roots only in the largest callus proliferations formed on decapitated stems treated with indoleacetic acid in lanolin, while according to SOLACOLU *et al.* similarly induced tumors 9–10 days old show root meristems, completely individualized, producing rootlets with limited

growth and abnormal development. The latter find that the tumor extends much farther down the stem than here reported, and that meristematic activity, except in direct contact with the paste, is localized in the meristematic zones, the cambium and pericycle. The results of the present experiment do not agree completely with this. Activity in the cortex extends at least half the length of the older tumor, activity of the pith extends sometimes the complete length of the tumor, and the endodermis is more meristematic in all regions than is the pericycle. They report extreme activity near the surface in older tumors but give no account of the vascularization of the endodermal derivatives reported in the present paper. They do, however, describe many tracheids differentiated in various regions near the cut surface.

The responses of *V. faba* to indoleacetic acid show marked similarities to the responses of other plants to similar treatments, although there are certain differences, notably in degree of response. As stated by HAMNER and KRAUS (13) for kidney bean, the results of this experiment indicate that any parenchymatous tissue of the stem of broad bean may become meristematic in response to treatment with indoleacetic acid and these cells later differentiate into vascular tissues. The epidermis forms an exception to this, but in old, very active tumors of *Vicia*, some epidermal cells may undergo division.

The slight surface callus formation of *V. faba* when treated with indoleacetic acid is different from the reaction of kidney bean (13), cabbage (9), tobacco (11), and sunflower (5) to the same treatment, while resembling somewhat the response of tomato (6), *Iresine* (14), pea (26), and *Mirabilis* (12). The regions of greatest activity vary from plant to plant and also with the growth substance used. The extreme activity of the cortex of broad bean resembles that of the kidney bean treated with naphthaleneacetic acid, except for the vascularization (13). Meristematic activity of the endodermis is found in many of the plants that have been treated; it is more extended in kidney bean (2, 13, 17, 22) than in broad bean. Increased activity of the fascicular cambium and development of an interfascicular cambium as seen in broad bean are characteristic of tumors of *Mirabilis* (12), and to a certain extent other plants treated with indoleacetic acid; but greatest development is reached, particularly as regards production of secondary xylem, in tumors of kidney bean induced by naphthalene acetamide (18). The pericycle, which is rather inactive in broad bean, tomato (6), cabbage (9), kidney bean (13, 17), and sunflower (5), is a region of great activity in *Mirabilis* (12) and *Iresine* (14), and is relatively active in pea (26). The phloem, which in broad bean is relatively inactive, is very active in kidney bean (2, 13, 17), pea (26), *Iresine* (14), and tomato (6). Divisions in the pith, so marked in *Vicia* tumors, are also found in others, as in *Mirabilis* (12), to a limited extent in tomato (6), in the formation of the callus in kidney bean (13), in limited regions of cabbage (9), and in sunflower (5). In other plants the pith remains relatively inactive, except close to the primary xylem strands. The relative inactivity of the epidermis is characteristic of all plants treated with indoleacetic acid, except of laterally treated stems of *Iresine* (14).

Irregular differentiation of vascular tissues is found in many of the tumors, but vascularization to the extent and

regularity characteristic of *V. faba* is not found except in the kidney bean tumors induced by tryptophane (16). The similarity between these two tumors in this and other respects is particularly interesting and needs further detailed consideration.

The greatest difference between the tumors produced by *V. faba* after treatment with indoleacetic acid and similarly produced tumors on other plants is the almost complete lack of root initiation. Kidney bean tumors induced by tetrahydrofurfuryl butyrate (25) and tryptophane (16) also show no root formation, but in other indoleacetic acid-induced tumors root formation is extensive and a characteristic feature of the tumor. The rather unorganized proliferations of *Vicia* correspond to nothing reported for other plants, unless such proliferations are interpreted as roots. If this is done the problem remains as to why the cells should have become vacuolate and nonmeristematic without complete differentiation and organization of tissues characteristic of roots. In other plants the root primordia, even though they may not develop far enough to break through the epidermis, remain meristematic and will resume activity if placed in a moist atmosphere. The occurrence of these structures leads to speculation as to the possibility of the existence of an organizing substance not present—or not produced in sufficient quantity—in *V. faba* for the development of normal roots.

The results of the present work, when compared with other similar work, would seem to suggest that both the degree and the type of response to any one growth-regulating substance, such as indoleacetic acid, varies with the species, each species offering a somewhat different internal environment. Certain of the investigations indicate that the effects vary in degree, particularly with variation in external environment, and in degree also with the age of the plant at time of treatment. This latter case has also proved true in *Vicia*.

Although polyploidy occurs to some extent in the tumors produced on the stem of *V. faba*, it seems improbable that it is specifically induced by the indoleacetic acid, although Greenleaf (11) considers the polyploid condition of shoots produced by decapitated tobacco plants to be induced by this acid and Levan (20) finds polyploid cells in abundance in roots of onion treated with indoleacetic acid and other growth substances. Dermen (8) reports polyploid cells in bean stems treated with naphthaleneacetic acid, but these are mainly in tissues likely to disintegrate subsequently. The potentiality for polyploidy is present normally in stems of *V. faba*, as shown by the scattered large nuclei and occasional polyploid figures in sections of control plants. It is conceivable that this tendency receives greater expression in treated plants because of the delay in maturation of and stimulation to increased activity in the cells in which the polyploidy occurs. A similar type of explanation might be extended to the case of tobacco, where callus does not normally form (10) but is induced by indoleacetic acid[1] and to the case of bean treated with naphthaleneacetic acid. The appearance of great numbers of polyploid cells in onion roots is more difficult to explain on a similar basis, although great numbers of such cells are found normally in roots of certain plants,

[1] Tomato produces polyploid shoots from a callus without the presence of the growth substance (21, 23).

for example, *Spinacia* (3), and moderate numbers in roots of certain legumes (28). However that may be, much more careful study is necessary before the specific relationship between growth-regulating substances and the possible induction of polyploidy can be established.

Summary

1. Plants of *Vicia faba* were decapitated just below the fifth node and the cut end treated with 1.5 per cent indoleacetic acid in lanolin or with lanolin alone.

2. The plants treated with indoleacetic acid responded by the formation of a tumor. The area, at most not more than 5 mm. long, below the treated surface became swollen and yellowed. There was little increase in size after the first 2 weeks following treatment.

3. The anatomy of the fourth internode of *V. faba* is similar to that in *Pisum sativum*, except for the number of bundles in the central cylinder.

4. Histologically every parenchymatous tissue became active to some extent in the formation of the tumor. Epidermal and outer cortical cells enlarged radially; the inner cortical cells divided transversely, occasionally the outer cortical cells tangentially; the endodermis divided tangentially; the cambium increased its activity and an interfascicular cambium was formed and became active as the early signs of response. Later parenchyma of the xylem, phloem, pericycle, pith, and rays became active to varying degrees. Cells of the pith divided repeatedly, and those bordering on the central cavity gave rise to an internal callus, the cells of which also divided repeatedly. In later stages all the cells of the cortex became highly meristematic. In some cases a callus was formed above the cut surface, starting from active cells of the vascular region and added to eventually by all parenchymatous cells. In the oldest tumors an occasional tangential division of the epidermis may be found.

5. Vascularization of derived tissues is somewhat extensive. Particularly notable is the response of the endodermal region of the central cylinder and of the cortical bundles. This group of extravascular strands may be supplemented by an occasional one formed in the pericycle or phloem. Small groups of wound tracheids are differentiated in the pith, pith callus, cortex, and surface callus when present. Occasionally vascular strands are formed in the pith close to the protoxylem points of the regular vascular cylinder.

6. Root induction, characteristic of some other plants treated with indoleacetic acid, is uncommon in *V. faba* under the conditions of this experiment. Those formed are probably derived as described for other plants. Occasionally, however, large, rather unorganized masses of tissue are developed which are probably best interpreted as potential roots incompletely organized.

7. Occasional polyploid nuclei are found in the large parenchymatous cells of the cortex and pith in both treated and control stems of *V. faba*, although somewhat more numerously in the treated stems. All divisions appear to be regular.

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